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THE PTOMAINES.

Their Forensic and Pathological Importance.

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THE PTOMAINES,

THEIR FORENSIC AND PATHOLOGICAL IMPORTANCE.

THE toxic effect of decaying animal matter has been known from the earliest times. The savages of uncivilized countries rendered their arrow-heads poisonous by steeping them in decaying carcasses, and many medical investigators have paid with health and life for their thirst for knowledge when making dissections with wounded hands. It was then already recognized that the source of the poison was inherent to the carcass, but it was not understood that the germs for its production were thus only transplanted, and that the toxic effect originated from their action on the new soil. It was reserved for the scientists of the last two decades to unravel the mysterious surroundings of their existence, and to recognize the chemical post-mortem changes to which they owe their presence. How vast since the days of Pasteur our views have been changed in regard to the nature of decay and putrescence, and the revolution created thereby in medicine, I scarcely need mention here. Sepsis and antisepsis prove beyond all doubt the chemical influence of the microbe. That such a complex molecule

as that of the albuminoids should be capable of being preserved intact when protected from the inroads of the almost ubiquitous micro-organisms would prove beyond doubt that its molecular decomposition, empirically termed decay and putrescence, is due solely and wholly to the influence of the bacterium. It tends to show, besides, that the organized body after death does not bear within itself the means of self-destruction and chemical decomposition.

In a methodical study of our subject there present themselves for our consideration three points,—to wit, 1. The agency, as the micro-organism ; 2. The material for its elaboration or soil ; and, 3. The one to which I will give my main consideration to-day,—their chemical products.

Various basic substances developed during the life of organized individuals owe their existence undoubtedly to chemical influences otherwise than those exerted by microbes ; such are the alkaloids of plants, and the leucomaines of Gautier, to be mentioned hereafter. From the dead tissues, however, no such products are developed, only by external agencies. It is a well-known fact that there are a great many distinct and different micro-organisms, as proven by their structure and form, as well as by their habits and products. The saccharomytes cerevisiæ cannot induce acetous, lacteous, or butyric fermentation any more than the bacterium thermo can split up glucose into alcohol, carbon dioxide, and water. Still, with the results attained by all of them the tendency remains the same, to disintegrate the complexer molecule into a

simpler one with by-products as various as are their different forms and the soils they act upon. This latter forms a very important and material part of our study, for while the first-mentioned germ may break up certain carbohydrates, it has no power to induce splitting up of nitrogenized matter. Thus, microbes can certainly not be considered as catalytic agents, as they must be able to utilize the soil they thrive upon as their natural pabulum, breaking up by hydration the molecule, rather than consuming it, and giving rise to the products thereof as excreta. To be able to appropriate to themselves part of the culture-material is one of the first principles for the disintegration of the latter, with the formation of simpler compounds in consequence thereof. Were the product a single one, it might be argued that it was simply excretory in character, but as the splitting up of the proteid molecule results in various products, the argument would favor the decomposition of the molecule by withdrawal of some of its component parts. The microbe given as a natural chemical factor, with the soil as containing material for its existence and propagation, the product would be dependent for its character upon their proper relation. Of these two, the basic products so derived constitute what are now known to us as "ptomaines," from ptoma, the fallen beast or carcass. To distinguish them from the similar results of vegetable life they are also called cadaveric alkaloids, or, better still, the alkaloids of decay.

The first to isolate an alkaloid from the tissues and fluids of the dead body were un-

doubtedly Dupré and Bence Jones, who extracted in 1866, by means of acidulating with sulphuric acid the masses operated on, separating the extract and neutralizing it, and then shaking it with ether, a basic substance. This was precipitable by iodine, iodohydrargyrate of potassium, phospho-molybdic acid, and the chlorides of gold and of platinum. On account of its fluorescence with sulphuric acid, it was called by them animal chinoidin. Sonnenschein and Zülzer separated in 1869 from liquids which had contained anatomical preparations, as well as from muscles and tissues, microscopical crystals, which gave the chemical reactions for atropine and hyoscyamine, which, when instilled into the eyes of rabbits and dogs, produced marked mydriasis, increasing the cardiac action when injected into the jugular vein of rabbits, and completely paralyzing peristalsis.

Rorsch and Fassender discovered in 1871, during a toxicological analysis, in which they employed the Staas-Otto method for the separation of alkaloids, a basic body in the liver, spleen, and kidneys, which gave alkaloidal reactions. Like digitalin, it gave a precipitate with phospho-molybdic acid which was gray, and turned intensely blue on addition of ammonia. Schwanert also separated about that time from a cadaver by the same method an alkaline fluid of the odor of trimethylamine, of bitter taste, forming crystals as a chlorate, and giving white vapors on addition of sodium hydrate, also answering many of the alkaloidal tests.

About the same time the now lamented Francesco Selmi brought out the importance

of the ptomaines for forensic medicine by his brilliant researches in a poison case, upsetting the expert testimony which tended to convict the accused. This was during the celebrated trial for the supposed murder by poison of the Italian general Gibbone, whose servant stood accused, on circumstantial evidence, of having administered to him the poison. Two prominent experts declared to have found delphinine in the intestines of the deceased. Selmi proved incontestably that the same reactions as produced by the experts for the prosecution would also result from a ptomaine obtained by him in the usual manner from the alkaline fluids of animal substances by extraction with ether. He showed, besides, that delphinine responded to many reactions which the substance derived from the deceased's body did not yield. Professors Ciaccia and Villa, of Bologna, demonstrated over and above this that the base obtained from defunct's body did not coincide in physiological action with that of delphinine.

This, of course, tended to revolutionize all dogmas in regard to poisoning by alkaloids, and the medico-legal proofs of their existence, especially as subsequently strychnine and morphine were also found to be closely simulated in their reactions by those of certain ptomaines.

In the case of the widow Sonzogna, in Cremona, whose body was exhumed twelve days after death, and where morphine was asserted to have been found, Selmi again, by an extended series of reactions and physiological experiments, proved without doubt that the supposed morphine was nothing but a cadaver alkaloid of similar chemical reactions.

The researches of the brilliant Italian toxicologist were soon confirmed from all quarters, and the ptomaines have to-day a place in forensic medicine which bids fair to upset all preexisting methods of investigation.

Liebermann separated from a decaying stomach, during a toxicological analysis, a base which possessed properties of coniine, but proved totally inert physiologically.

During the case of Brandes-Krebs, tried before the assizes of Brunswick in 1884, there was discovered, besides arsenic, a coniine-like body, which corresponded in reaction both with coniine and nicotine, but differed in some reactions from both. It was very poisonous, 44 milligrammes killing a frog within a minute.

Another coniine-like base was isolated by Brouardel and Boutmy from the body of a woman, who, with others, had eaten of a stuffed goose. The same base could be obtained from the remnants of the goose. It gave the general alkaloidal reactions and others non-alkaloidal besides, and also acted as a poison on frogs. The same investigators also isolated from other dead bodies a veratrine-like body, which reduced potassium ferricyanide, and did not induce tetanic symptoms in frogs. They proposed as general reactions for the ptomaines the reduction of potassium ferricyanide, maintaining that none of the vegetable alkaloids aside of morphine possessed this property in a marked degree. Gautier and others showed, however, that it was also found with hyoscyamine, emetine, igasurine, colchinine, nicotine, and apomorphine, as well as ergotinine, aconitine, digitalin, eserine, and

some of the other opium alkaloids. The poisonous properties of the ptomaines were proven by Moriggia and Battistina in 1875, and with it the value of the physiological experiment in their differentiation with vegetable alkaloids, which was urged by Ranke as being even more conclusive than chemical tests.

Selmi showed in 1876 that the duration of the decay had a marked influence on the production of the ptomaines, so that *different bodies are generated at different times, and that all of them are unstable.* In his celebrated monograph, "Sulle ptomaine od alcaloidi cadaverici e loro importanza in tossilogia," Bologna, 1878, he maintains materially the Staas-Otto method for the separation of ptomaines, and classes them as follows, according to their extraction by ether: 1. Those from acid fluids; 2. Those from alkaline fluids; then, by their extraction with chloroform, from, 3. Acid fluids; 4. Alkaline fluids; 5. Those which are extracted by amylic alcohol; and, 6. Those remaining in the extracted masses. He further gives the principal reactions for the products of each of these classes, and dwells particularly upon the fluid coniine-like ptomaines. He compares with them the principal vegetable alkaloids which simulate them, showing that morphine and codeine, as well as atropine and delphinine, are particularly prone to such simulation by ptomaines.

His work on the recognition of and the differentiation between morphine and its simulating ptomaine has since become a matter of record. The results of his vast labors on the ptomaines, the study of which he made

his life's work, Selmi comprised in a work entitled "Ptomaine od alcaloidi cadaverici e prodotti analoghi da certi malatti in correlazione colla medicina legale," Bologna, 1881. His researches on the products of decay of egg albumen demonstrated the development of several non-volatile basic substances, the hydrochlorides of which crystallized in colorless needles, and acted upon frogs like curare. They were also directed towards defining the conditions influencing the development of ptomaines, so that without access of air they were found to develop only ammonia, while under the influence of the atmosphere amines were formed. If these were treated with fixed alkalies there resulted bodies of an intense coniine odor.

While in Selmi we have no doubt lost the most energetic worker on the subject of ptomaines, others, contemporary with him and after him, added to our stock of knowledge in this direction. To-day their existence forms one of the formidable barriers to medico-legal proofs, and complicates the work of the toxicologist in the most embarrassing manner. Not alone that many of them have poisonous properties in themselves, but they are capable besides of forming poisonous compounds with inorganic poisons. To swear away a man's life under such circumstances is a grave responsibility indeed.

The importance of these substances in forensic medicine appears clearly from some of the here briefly alluded to references. While no doubt few of the alkaloids and ptomaines cannot be distinguished by corroborative chemical tests and physiological experiment,

the danger is still possible that some alkaloid may be identical with a ptomaine. That animal bodies are not the only substances capable of producing ptomaines will become evident from the fact that they will form wherever proteids are subject to decay,—*i.e.*, exposed to the action and decomposing influence of micro-organisms. Thus, a crystalline poisonous ptomaine, termed sepsine, was isolated from decaying yeast by Bergmann and Schmiedeberg, while a body giving the color reaction of strychnine, but without bitter taste, was separated from putrescent corn-meal; and Poehl, as one of a commission of the Russian government to investigate the cause of epidemic ergotism, found ptomaines in large quantities in the ergotized flour.

But it is not to forensic medicine alone that a knowledge of the ptomaines is of such great importance. Their existence has opened up to the pathologist a vista which promises to solve the mysterious nature of infectious diseases. It may, indeed, be claimed that science moves in cycles, and that in our search for knowledge we return to the old and cast-away hypotheses, to view them with eyes fortified in a better manner. Humeropathology, long since passed away from modern medical science, seems to be recalled to us, though in an entirely new garb. The study of the physiological action of poisons proves beyond doubt that their toxic influence is proportionate with the chemical constitution of the intoxicant. The study of the nature and action of the microbes has forced more and more upon us the condition that their presence and mechanical irritation alone

could in no way account for the different types of disease, and that they must manifest themselves rather by chemical effect than by simple physical traumatisms. Even by the most sceptic the presence of special types of micro-organisms in different diseases in countless numbers cannot be denied, while their absence in health is also established. The principal doubt seems to have been always as to their pathogenetic function. It is not to be denied that they are as such not pathogenetic, that they may exist in limited numbers or on unsuitable soil without producing disease. That they are organized individuals, depending for their life and propagation on chemical changes, none will or can dispute. Organized individuals require for their natural life food, and the foods differ in the plants and animals almost as much as do the characters of their different species. The food is not merely consumed as such and ejected, but the vital phenomena are but the results of the chemical changes they undergo in the consumer, the heat units stored up therein being definitely reproduced either in mechanical action, heat radiation, or in reproduction and other vital functions. While in the micro-organism we can scarcely claim for this correlated force production many of the familiar vital phenomena, their rapid reproduction certainly shows that their food consumed is not an unimportant matter. While only a limited portion, however, is utilized in the reproduction of force, the greater portion of the molecule broken up by them, now disassociated, seeks other relations, and furnishes new products which are foreign to the animal organism.

Given the presence of the micro-organism and the disease proportionate in its intensity to the numerical strength of the former, together with an array of symptoms not to be explained by simple mechanical irritation only, what is left to account for the typical character of disease? While this may be said to be arguing for the presence of pathogenetic matter by exclusion, it is by no means intended as proof, which the chemical pathologist of the present has already furnished in some instances, and will, no doubt, further furnish in the near future. The late Austin Flint foresaw this when he intrusted the future of medicine to chemistry, and daily the evidence is accumulating that infectious diseases are but specific intoxications with products resulting from special micro-organisms.

The study of the ptomaines, not as carriers of, but as specific intoxicants during, disease, is one, therefore, that offers a wide field of action for chemical pathology. As chemistry is a concise science, the mere reactions of complex bodies, such as we have referred to under the forensic part of this paper, will hardly suffice, and their ultimate composition alone with the atomic arrangement of their molecule is what the chemists will have to prove before we can hope to throw light on the changes they exert on the normal vital process.

Although many of the previously-mentioned investigators had isolated crystallizable and crystallized ptomaines, these were not in sufficient quantities to admit of their elementary analysis. Many of the others were even complex bodies, which owed some of

their characteristic reactions to admixed products.

To Nencki, of Berne, we owe the first knowledge of the composition of a chemically pure ptomaine, the "collidine," $C_8H_{17}N$, a homologue of the pyridines, which he obtained as a double platinum salt, while subsequently Gautier and Etard prepared from decaying mackerels the ptomaine "parvoline" of the chemical composition $C_9H_{13}N$, another homologue of the pyridine group.

Brieger, of Berlin, took up the study of the ptomaines with great energy, and we owe to him and the analytical chemists he surrounded himself with much of our present chemical knowledge on this subject. His researches were first directed to the aromatic products of the decomposition of egg albumen, with special inquiry into the elements of their formation. These consist—1. In the nature of the ferment; 2. The manner in which albumen is offered to putrescence; 3. The temperature influencing it; and, 4. The part oxygen takes in the process. The production of phenol, cressol, indol, and skatol, and their derivatives resulted from these researches, which were confirmed again by himself and Baumann. E. and H. Salkowski further proved the formation of aromatic acids during putrescence, while Schmiedeberg and Harnack showed that neurine by oxidation may be converted into the poisonous muscarine. Brieger proved that peptones and peptotoxin were identical with many of the poisonous bases of albuminoid decay. To enumerate here the various researches of Brieger would lead us too far into the domain of physiologi-

cal chemistry and from the object of this paper. Let me condense his results, especially of the cadaver ptomaines, in his own words, that "during the various stages of decay of the human cadavers and their parts, different basic products are formed; many ptomaines disappear during the process and others form in their place, and, as in the beginning, certain bases are quite rare, with the partial disappearance of others the first formed reappear in a greater degree." With cessation of life in the animal body, the complex lecithin is broken up into its constituents, of which cholin is the most marked. Within about the third day a diamine, termed by Brieger "neuridine," appears; after about seven days cholin disappears, and with its disappearance markedly toxic ptomaines develop. Another diamine, similar to neuridine, of the formula $C_5H_{16}N_2$, was found, and termed cadaverine, and appeared more plentiful the longer putrescence had lasted. A third diamine, at a still more protracted putrescence, was found by Brieger to have the formula $C_4H_{12}N_2$, and termed putrescine. Still a fourth diamine, a polymere of neuridine, he termed saprine, all of which, however, proved without toxic action. Of the only two poisonous bases that Brieger could separate from the human cadaver the one producing toxic properties in the most marked degree he terms mydaeline, which produced mydriasis and death in small animals. While thus many of the cadaveric bases of Brieger's are principally diamines, and not related to the pyridine series of alkaloids, they appear to belong to the ethylenes, and do not correspond to the reduction test

of potassium ferricyanide, proposed as a general reagent for all ptomaines by Brouardel and Boutmy. Thus, no group reagent for cadaveric bases exists, although it is maintained that while alkaloids are optically active, the ptomaines are optically inactive. Still, as they are obtained in such small quantities, this property, were it even proven to belong to the ptomaines as a class, could be hardly available. To obtain a ptomaine for forensic practice it would from the preceding appear necessary to prepare it absolutely pure, and compare its chemical composition with the supposed alkaloid. For the detection of pathological ptomaines it is proposed to obtain from the body the characteristic microbe, and from artificial cultures study its product. If the theory that pathological ptomaines are of an excretive nature were true, the results would have to be correct, but if the ferments, according to chemical theory, are agents of hydration only and splitting up of the molecule, there can certainly be no comparison between that of artificial culture-fluid and that of the albuminoids of the body. Still, if the ptomaines correspond in physiological action to the clinical picture of the disease they may be inferentially accepted as identical.

A ptomaine of cholera was isolated in 1885 by Villiers; it was principally found in the intestines, and in traces in the kidneys, but not in the heart or liver. It had an odor of trimethylamine, was volatile, and had a biting taste. It answered the usual ptomaine reaction, and acted on animals by creating nervous tremor, and in making the cardiac beat intermittent. He also separated from the

organs of a child dead from pneumonia a volatile, acrid-smelling ptomaine, differing in its physiological action and by its reactions from the cholera ptomaine, and with feeble basic properties. This base he considered identical with the ptomaine from a child which had died of diphtheria.

Armand Gautier is also one of the prominent investigators on this subject. He objects to the Dragendorf method for their separation, and also to that of Pouchet by precipitation with tannin. He defends their structure as true alkaloids against Casali, and gives parvoline, already mentioned, and hydrocollidine, $C_8H_{13}N$. Both these bases are fluid, and from the mother-liquor, after precipitation with platinum chloride, another volatile base of the odor of elder-flowers is obtained. He describes the ether, chloroform, and amylic alcohol extracts of cadavers, and states that they act similar to muscarine, while the hydrocollidine killed a bird in an hour, with accompanying muscular paralysis. He describes as leucomaines the bases derived from the normal tissues of living animals which are derivates of kreatin, kreatinin, and xanthin. Gautier infers from his experiments, that from the similarity of ptomaines and leucomaines, the tissue-metamorphosis of the body is similar to ærobic and anærobic micro-organisms; that the excretion of injurious products is partly effected by *mechanical* means and partly by *oxidation*. This important function of oxygen is, as he agrees with Bouchard, of special value in pathological conditions, when the products of tissue-metamorphosis accumulate in the system.

Gautier further argues that, as the poisonous nitrogenized extractives of the urine possess no basic properties, the injurious products within the body are not alkaloids.

In contradistinction to the just quoted celebrated author, Brieger, in his third publication on the ptomaines (Berlin, 1886, Hirschwald), lays particular stress on the chemical action of the micro-organisms, and states that their valuable work in nature's workshop is not sufficiently appreciated. For the poisonous ptomaines he proposes the affix toxines. Besides cadaverine and putrescine, he separated from decaying horseflesh a poisonous amido-acid, $C_7H_{17}NO_2$, also mydatoxine, $C_6H_{13}NO_2$, which produced symptoms like curare. He also isolated mydine, $C_8H_{11}NO$, and methylguanidine, $C_2H_7N_3$. He made special investigations on the ptomaines of pure cultures of pathogenetic bacteria. Thus, he found that the staphylococcus pyogenes aureus, Rosenbach, apparently produces no poisonous ptomaine and only salts of ammonium ; that neither normal flesh parts, nor xanthin or kreatin, were attacked by it or the streptococcus pyogenes, Rosenbach, which produced, besides ammonium chloride, principally trimethylamine. From the cultures of the Koch-Eberth typhoid bacillus on meat pulp, he obtained the basic typhotoxine, $C_7H_{17}NO_2$, which induced in small animals lethargic conditions, with liquid dejecta. This bacillus, while easily decomposing the carbohydrates, does not affect glycogen. Cultures with tetanus ferment, though not sufficiently purified, developed in a hydrogen atmosphere a base of the composition of $C_{13}H_{30}N_2O_4$, which

physiologically produced the same symptoms as had been before obtained with the cultures.

I have myself, at the instance of Dr. Shakespeare, extracted from the brain of a mule which had died of tetanus a basic substance by the Staas-Otto method, which possessed tetanizing properties.

I cannot close my account of pathological ptomaines without referring to the one discovered in this country by Vaughan, of Michigan. To this he gave the name of tyrotoxicon, from its origin from putrid cheese. This, while developed at first principally to clear up the frequent poisoning from dairy products, was by its discoverer inferentially declared to be the pathological ptomaine of cholera infantum. While as yet the proofs to this end are not conclusive, the proofs by analogy offered by Vaughan, together with the clinical experience in such cases, leave no doubt that tyrotoxicon is really the ptomaine producing the symptoms of that disease. In his interesting paper, read before the Section of State Medicine at the thirty-eighth annual meeting of the American Medical Association, the author proved beyond much doubt the identity of tyrotoxicon with diazobenzol, $C_6H_5N_2C_4H_7O_2$, both by chemical as well as physiological tests. The important bearing of this discovery on the milk-feeding of infants during our heated term is, indeed, a great one, and has already borne fruitful results.

As an agent that has caused frequent forensic investigations, tyrotoxicon has cleared up many mysterious poisoning cases that were formerly attributed to wilful admixtures. I

have had several opportunities of verifying its presence where charges of poisoning by arsenic had been made. The same may be said of other ptomaines which were the poisonous elements in chicken and lobster salads, canned meats and fish, as well as sausages, when wholesale poisoning was promptly attributed to other agents admixed to the viands.

Before concluding this account of the ptomaines, which, though extended, is still too brief to do justice to the subject, I would give a short *résumé* of that which I have presented to you, with inferences as have occurred to me during their study.

That there are poisonous products developed during the decay of albuminoids is undeniable. That decay depends upon the chemical agency of micro-organisms is probably now a scientific fact, only doubted by those who are irreconcilable to scientific advance. That these products of decay bear a strong resemblance in their effect on the living body to the alkaloidal substances of vegetable origin is also well confirmed, though chemically no similarity appears to exist. That they are transient in their origin would point to their instability as chemical compounds.

When reasoning with these points as basis, the conviction forces itself upon us that the chemistry of the ptomaines is one of great complexity,—*i.e.*, that they cannot as a whole be classed with a group or series, and even the ingenious researches of the ablest investigators have not succeeded in elucidating it sufficiently; also that even great care and

skill will not always prevent chemical new formations during their isolation ; and again, the processes employed for their purification and isolation in a pure state appear to show a tendency to reduce their complex molecule to simpler forms. The chemical process alone without physiological experiment is not always reliable in demonstrating their nature or character. Their development from dissimilar soils cannot, in my opinion, be accepted as the specific products of the bacterium from the living albuminoid molecule. The fact that basic substances are not found in the urine, as is the case with vegetable alkaloids, seems to me no argument either against their existence in the blood or for their destruction by oxidation in the body. The majority, if not all, the ptomaines are in their basic state volatile substances. From the alkaline liquids of the body they would thus most likely find their elimination rather through the respiratory organs than the skin or kidneys. The poisonous properties of the ammoniacal compounds of the body in the pulmonary exhalations are well known, and, in my opinion, the pathological ptomaines find their exit from the circulation by the lungs, which I hope to be able to prove in the near future from experiments which I am conducting at this present time.

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